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UTILITY PATENT APPLICATION TRANSMITTAL	Attorney Docket No S-91,714
	First Inventor or Application Identifier Arlene A. Wise
	Title: DETECTION OF PHENOLS USING ENGINEERED BACTERIA
	Express Mail Label No: EI559797827US

APPLICATION ELEMENTS	ADDRESS TO: Box Patent Application Assistant Commissioner for Patents Washington, DC 20231
1. <input checked="" type="checkbox"/> * Fee Transmittal Form (e.g. PTO/SB/17) (submit an original and a duplicate for fee processing) 2. <input checked="" type="checkbox"/> Specification [Total Pages 29] <input checked="" type="checkbox"/> Descriptive title of the invention <input checked="" type="checkbox"/> Cross References to Related Applications <input checked="" type="checkbox"/> Statement Regarding Fed sponsored R&D <input type="checkbox"/> Reference to Microfiche Appendix <input checked="" type="checkbox"/> Background of the Invention <input checked="" type="checkbox"/> Brief Description of the Drawings (if filed) <input checked="" type="checkbox"/> Detailed Description <input checked="" type="checkbox"/> Claim(s) <input checked="" type="checkbox"/> Abstract of the Disclosure 3. <input checked="" type="checkbox"/> Drawings(s) (35 U.S.C 113) [Total Sheets 07] 4. <input type="checkbox"/> Declaration & Power of Attorney [Total Pages] a. <input type="checkbox"/> Newly executed (original or copy) b. <input type="checkbox"/> Copy from a prior application (37 C.F.R. § 63(d)) (for continuation/divisional with Box 16 completed) c. <input type="checkbox"/> DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33 (b). 5. <input type="checkbox"/> Microfiche Computer Program (Appendix) 6. Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary) a. <input type="checkbox"/> Computer Readable Copy b. <input type="checkbox"/> Paper Copy (identical to computer copy) c. <input type="checkbox"/> Statement verifying identity of above copies	ACCOMPANYING APPLICATION PARTS 7. <input type="checkbox"/> Assignment Papers (cover sheet & documentation) 8. <input type="checkbox"/> 37 C.F.R. § 3.73(b) Statement (when there is an assignee) <input type="checkbox"/> Power of Attorney 9. <input type="checkbox"/> Information Disclosure Statement (IDS)/PTO-1449 <input type="checkbox"/> Copies of IDS Citations 10. <input type="checkbox"/> Preliminary Amendment 11. <input checked="" type="checkbox"/> Return Receipt Postcard (MPEP 503) (should be specifically itemized) 12. <input type="checkbox"/> Small Entity Statement(s) <input checked="" type="checkbox"/> Statement filed in prior application Status still proper and desired 13. <input type="checkbox"/> Certified Copy of Priority Document(s) (if foreign priority is claimed) 14. <input type="checkbox"/> Other:

15. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in a preliminary amendment

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application S.N. ____/____.

Prior application information: Examiner

Group/Art Unit:

For CONTINUATION or DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 4b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

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Docket No.: S-91,714

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Art Unit:

For : DETECTION OF PHENOLS USING ENGINEERED BACTERIA

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Country	Year	Population (millions)	Urban population (millions)	Urban population (%)	Population density (per sq km)	Population density (per sq mi)
Algeria	1980	10.0	4.0	40.0	100	260
Algeria	1985	10.5	4.5	42.9	105	272
Algeria	1990	11.0	5.0	45.5	110	284
Algeria	1995	11.5	5.5	47.8	115	297
Algeria	2000	12.0	6.0	50.0	120	310
Algeria	2005	12.5	6.5	52.0	125	323
Algeria	2010	13.0	7.0	53.8	130	336
Algeria	2015	13.5	7.5	55.6	135	349
Algeria	2020	14.0	8.0	57.1	140	362
Algeria	2025	14.5	8.5	58.6	145	375
Algeria	2030	15.0	9.0	60.0	150	388
Algeria	2035	15.5	9.5	61.3	155	401
Algeria	2040	16.0	10.0	62.5	160	414
Algeria	2045	16.5	10.5	63.6	165	427
Algeria	2050	17.0	11.0	64.7	170	440
Algeria	2055	17.5	11.5	65.7	175	453
Algeria	2060	18.0	12.0	66.7	180	466
Algeria	2065	18.5	12.5	67.6	185	479
Algeria	2070	19.0	13.0	68.4	190	492
Algeria	2075	19.5	13.5	69.2	195	505
Algeria	2080	20.0	14.0	70.0	200	518
Algeria	2085	20.5	14.5	70.7	205	531
Algeria	2090	21.0	15.0	71.4	210	544
Algeria	2095	21.5	15.5	72.1	215	557
Algeria	2100	22.0	16.0	72.7	220	570
Algeria	2105	22.5	16.5	73.3	225	583
Algeria	2110	23.0	17.0	73.9	230	596
Algeria	2115	23.5	17.5	74.5	235	609
Algeria	2120	24.0	18.0	75.0	240	622
Algeria	2125	24.5	18.5	75.5	245	635
Algeria	2130	25.0	19.0	76.0	250	648
Algeria	2135	25.5	19.5	76.5	255	661
Algeria	2140	26.0	20.0	76.9	260	674
Algeria	2145	26.5	20.5	77.4	265	687
Algeria	2150	27.0	21.0	77.8	270	700
Algeria	2155	27.5	21.5	78.2	275	713
Algeria	2160	28.0	22.0	78.6	280	726
Algeria	2165	28.5	22.5	78.9	285	739
Algeria	2170	29.0	23.0	79.3	290	752
Algeria	2175	29.5	23.5	79.7	295	765
Algeria	2180	30.0	24.0	80.0	300	778
Algeria	2185	30.5	24.5	80.3	305	791
Algeria	2190	31.0	25.0	80.6	310	804
Algeria	2195	31.5	25.5	81.0	315	817
Algeria	2200	32.0	26.0	81.3	320	830
Algeria	2205	32.5	26.5	81.6	325	843
Algeria	2210	33.0	27.0	81.8	330	856
Algeria	2215	33.5	27.5	82.1	335	869
Algeria	2220	34.0	28.0	82.4	340	882
Algeria	2225	34.5	28.5	82.6	345	895
Algeria	2230	35.0	29.0	82.9	350	908
Algeria	2235	35.5	29.5	83.1	355	921
Algeria	2240	36.0	30.0	83.3	360	934
Algeria	2245	36.5	30.5	83.6	365	947
Algeria	2250					

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CROSS-REFERENCE TO RELATED APPLICATIONS

This nonprovisional patent application claims the benefit of copending provisional patent application No. 60/123,659 which was filed on March 08, 1999.

FIELD OF THE INVENTION

5 The present invention relates generally to the detection of organic pollutants and, more particularly, to the mutagenic generation of a group of DmpR protein derivatives with improved ability to activate transcription of a reporter gene in bacteria in the presence of phenols, including certain disubstituted phenols, in liquids and soils. This invention was made with government support under Contract No. W-7405-ENG-36
10 awarded by the U.S. Department of Energy to The Regents of The University of California. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

15 In the last three decades, there has been a significant increase in government regulations that hold industrial entities accountable for the chemical pollution that results from their manufacturing activities. In order to comply with environmentally sensitive regulations, businesses must be able to identify contamination and monitor its remediation processes. The cost and technical complexity of chromatographic methods currently in use may act to limit characterization of contaminated sites. One way to lower the cost of detection is to use biosensors derived from genetic systems of bacteria
20 that have evolved to use organic contaminants as growth substrates.

25 Certain strains of soil bacteria have evolved the capacity to use toxic organic chemicals as food sources. Production of the required metabolic enzymes is, in some cases, controlled by a particular type of regulatory protein that detects the toxic chemical through a direct physical interaction. The protein-chemical complex binds to a cognate promoter sequence and activates expression of genes encoding the required metabolic enzymes. This type of regulatory protein can be utilized as a pollution detecting component in bacteria that have been engineered to signal the presence of environmental pollution.

The most basic whole cell bacterial biosensors are created by placing a reporter gene under control of an inducible promoter. Expression of the reporter gene provides a measurable signal when the appropriate transcription activator protein interacts with an effector chemical.

5 Phenol and various substituted phenols are used in the manufacture of dyes, photographic chemicals, pesticides, lumber preservatives, microbiocides and herbicides. Current methods for detecting phenol contaminants include gas chromatography and high-pressure liquid chromatography. These chromatographic methods require expensive equipment and highly trained technicians. In response to
10 the U.S. Environmental Protection Agency having listed eleven phenols as priority pollutants, industries that use phenol and phenol derivatives require simple and inexpensive detection methods to identify spills, leaks, and other phenol contamination that result from their manufacturing and service activities.

The construction of bacterial biosensors is limited by the restricted availability of
15 bacteria that are known to metabolize a chemical of interest and, in particular, by the absence of knowledge concerning the genetic systems that control bacterial response to the chemical. Fortunately, some of the bacterial genetic systems that support metabolism of polluting chemicals show significant genetic relatedness. Operons encoding genes required for metabolism of phenol, toluene, benzene, and xylene in
20 some *Pseudomonas* and *Acinetobacter* species are headed by promoters recognized by sigma-54-associated RNA polymerase. Transcription directed by these promoters occurs when the system's regulatory protein detects the presence of the substrate for the catabolic enzymes. Proteins in this category include DmpR, XylR, MopR, PhhR, PhIR, and TbuT. These six proteins show significant similarity to one another at the
25 amino acid level. Sequence information and domain swapping experiments indicate that the general arrangement of these regulatory proteins consists of discrete areas with three independent functions including chemical detection, polymerase activation, and DNA-binding.

XylR and DmpR are the most studied members of this group of transcription
30 activators. The *Pseudomonas putida* XylR has already served as the detection component for a number of biosensors based on its ability to activate transcription in

response to xylene, toluene and benzene. DmpR, the product of the *Pseudomonas* CF600 *dmpR* gene, mediates expression of the *dmp* operon to allow growth on simple phenols. Transcription from *Pdmp*, the promoter heading the *dmp* operon, is activated when *DmpR* senses the presence of phenol, cresols, mono-chlorinated phenols, and some mono-methylated phenols (See, e.g., V. Shingler and T. Moore, "Sensing of aromatic compounds by the DmpR transcriptional activator of phenol-catabolizing *Pseudomonas* sp. strain CF600", J. Bacteriol. **176**:1555-1560 (1994)). Disubstituted phenols, such as 2,4-dichlorophenol or 2,4-dimethylphenol, are inferior inducers of *dmp* transcription.

Domain swapping experiments to form XylR-DmpR hybrids demonstrated that the sensor activity of these regulatory proteins is localized to the amino terminal region. By switching the first 234 amino acids of DmpR with those from XylR, Shingler and Moore, *supra*, created a chimeric protein that activated transcription from *Pdmp* in response to toluene and xylene, but not phenol or cresol. The results of the hybrid protein experiments indicated that transcription from *Pdmp* depends on a direct physical interaction between the sensor domain of DmpR and the inducing phenol.

The single regulatory protein, and the independent domain arrangement of DmpR and other proteins of this type make them particularly suitable candidates for genetic manipulation and suggests a way around the restrictions imposed by limited information about the genetics that control bacterial degradation of xenobiotics. Such altered proteins have the potential to extend the chemical target range of biosensors beyond that based on natural systems.

Therefore, it is an object of the present invention to alter the chemical sensing domain of the protein DmpR to respond to phenol and phenol derivatives which are poorly detected or undetected by the wild type protein.

Another object of the invention is to alter the chemical sensing domain of the protein DmpR to respond to phenol and phenol derivatives without disturbing its transcription activating functions.

Additional objects, advantages and novel features of the invention will be set forth in part in the description which follows, and in part will become apparent to those skilled in the art upon examination of the following or may be learned by practice of the

invention. The objects and advantages of the invention may be realized and attained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

SUMMARY OF THE INVENTION

To achieve the foregoing and other objects, and in accordance with the purposes of the present invention, as embodied and broadly described herein, the method for enhancing bacterial response to organic molecules, where the bacteria have a regulatory protein with discrete functional domains for independent activities, one such domain being a sensor domain that detects the organic molecules through a direct physical interaction forming a protein-molecule complex which binds to a cognate promoter sequence and activates expression of genes encoding metabolic enzymes, includes modifying the sensor domain of the regulatory protein such that the response to the organic molecule is enhanced without altering the other domains.

Benefits and advantages of the present invention include the creation of a large variety of engineered proteins with abilities to detect toxic organic chemicals. Such engineered proteins will be useful in development of environmentally beneficial tools that both detect and degrade polluting chemicals.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and form a part of the specification, illustrate an embodiment of the present invention and, together with the description, serve to explain the principles of the invention. In the drawings:

FIGURE 1 shows the organization of the DmpR protein and illustrates the domains having distinct functions which made it possible to modify the protein's sensor domain to change its capacity for interacting with a particular phenol without destroying its ability to bind DNA and activate transcription from its cognate promoter *Pdmp*.

FIGURE 2a shows the results of a β -galactosidase assay for the detection of 2-chlorophenol, while FIG. 2b shows the assay for 2,4-dichlorophenol using the bacterial test strain containing either wild type DmpR or the derivative DmpR-B21, where NI indicates a negative control containing no inducer (no phenol).

FIGURE 3a shows assays comparing wild type DmpR and DmpR-B23 as detectors for six concentrations of 2-chlorophenol after a 60 minute exposure, while

FIG. 3b shows assays comparing the response of wild type DmpR and DmpR-B17#2 to six concentrations of 4-chloro-3-methylphenol.

FIGURE 4a shows that a four-hour exposure to phenols increases the detection signal (in β -galactosidase activity units) for low concentrations of 2-chlorophenol for DmpR-B23, whereas FIG 4b shows the response of DmpR-B17#2 to low concentrations of 2,4-dichlorophenol, 2-nitrophenol, and 4-nitrophenol.

FIGURE 5a compares the detection of 2,4-dichlorophenol by DmpR-B9 with that of the wild type DmpR for unsubstituted phenol shown in FIG. 5b in contaminated soil, unsubstituted phenol being a natural effector of wild type DmpR.

FIGURE 6a compares the detection of 2-chlorophenol, 2,4-dimethylphenol, and 4-nitrophenol by wild type DmpR with that for the engineered protein DmpR-B31, while FIG. 6b compares the detection of 2,4-dichlorophenol and 4-chloro-3-methylphenol by the wild type DmpR and DmpR-D12; DmpR-B31 showing a strong response to most substituted phenols, but having a relatively high uninduced (NI) value.

FIGURE 7a compares the detection of 2-chlorophenol by DmpR-D9 with its detection by wild type DmpR, while FIG. 7b compares the detection of 2-nitrophenol by DmpR-D9 with that for wild type DmpR.

DETAILED DESCRIPTION

Briefly, the present invention includes a method for creating mutant DmpR derivatives having increased response to phenol and substituted phenols. These mutant derivatives are shown to activate the transcription of a reporter gene in the presence of the seven phenols listed as priority pollutants by the U.S. Environmental Protection Agency. Codon changes, including three silent mutations, that improve DmpR's ability to detect disubstituted phenols including 2,4-dichlorophenol and 2,4-dimethylphenol are described for both liquid and soil assays. Additionally, the mutations improve the ability of DmpR to detect di-substituted 4-chloro-3-methyl phenol and mono-substituted 2-chlorophenol, 2-nitrophenol, and 4-nitrophenol. Regulatory proteins capable of detecting organic contaminants through a specific physical interaction have been identified in some *Pseudomonas* and *Acinetobacter* species. Proteins of this type include DmpR, XylR, MopR, PhhR, PhIR, and TbuT. The general arrangement of these proteins consists of discrete functional domains with independent activities. The highly

conserved carboxyl and middle regions of these proteins contain regions dedicated to DNA-binding and transcription activation. The less-conserved amino terminal region is known as the sensor domain and is the portion of the protein that physically interacts with (detects) a specific chemical. For example, DmpR, MopR, PhhR, and PhlR detect phenol and activate production of enzymes that metabolize phenol. Chemicals detected by XylR and TbuT include toluene and xylene.

Because of their distinct functional domain organization, proteins of this kind are particularly suitable to genetic modification. A key element in the present invention is the mutagenic modification of DNA corresponding to a protein's sensor domain because this region can be mutagenized without changing the protein's ability to bind DNA and activate gene expression from its cognate promoter. Modification of a sensor domain has potential for creating novel proteins with altered or improved chemical detection ability.

Mutations in the sensor domain of DmpR were generated through mutagenic PCR. Engineered genes were transformed into bacterial test strains that carried DmpR's cognate promoter fused to a reporter gene. This test strain allowed identification and characterization of novel versions of DmpR with chemical detection capabilities that significantly differ from that of the wild type protein. Engineered derivatives of DmpR detect 2,4-dichlorophenol and 2,4-dimethylphenol, as well as other phenolic molecules that are not detected by wild type DmpR.

Reference will now be made in detail to the present preferred embodiments of the present invention, examples of which are shown in the accompanying drawings. Turning now to Fig. 1, the *dmpR* wt-N gene (See, e.g., Shingler and Moore, *supra*) carried on plasmid pAW50 served as the template for amplification of the *dpmR* sensor domain by mutagenic polymerase chain reaction (PCR). The products resulting from the mutagenic PCR were digested using NdeI and SacII and ligated back into a pAW50 plasmid fragment, from which the wild type NdeI-SacII region had been removed. This procedure resulted in replacement of DNA corresponding to the first 175 amino acids of DmpR (about 85% of the sensor domain). Ligation products were electroporated into AW101 (*trp::Pdmp-lacZ* fusion). Transformants were initially selected for tetracycline resistance and then replicated onto plates containing X-gal and one of the test phenols.

Colonies that developed more blue color than colonies containing wild type DmpR were selected for further analysis with liquid β -galactosidase assays. β -galactosidase activity is proportional to transcription of the *Pdmp-lacZ* reporter fusion and is, therefore, a measure of particular variant DmpR's ability to detect phenol or specific substituted phenols. This mutagenic procedure led to the identification of more than twenty DmpR derivatives with altered response to phenols. Five of these derivatives are representative of proteins which show significant promise as effective detectors of phenols listed as primary pollutants by the U.S. Environment Protection Agency.

Having generally described the invention, the following EXAMPLE provides additional details.

EXAMPLE

A. Bacterial strains and plasmids. *E. coli* TE2680 (Elliot, T. 1992. "A method for constructing single-copy *lac* fusions in *Salmonella typhimurium* and its application to the *hemA-prfA* operon," J. Bacteriol. **174**:245-253.) was used as an intermediate strain for placing the *Pdmp-lacZ* fusion into the chromosome of *E. coli* MC4100 (Casadaban, M. 1976. "Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage, lambda and Mu," J. Mol. Biol. **104**:541-555.) to create the *dmpR* test strain AW101. DH5 α (Sambrook, J. E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual, 2nd Edition. Cold Springs Harbor Laboratory Press. Plainview, New York.) was host for plasmid constructions.

pVI401 (See, e.g., Shingler and Moore, *supra*) served as the source of both the *dmpR wt-N* gene and the *Pdmp* promoter which heads the divergently transcribed *dmp* operon. *dmpR wt-N* contains a synthetic *NdeI* restriction site resulting from nucleotide changes immediately upstream from the ATG initiation codon. The coding region of *dmpR wt-N* remains the same as that of wild type *dmpR* and the response of the encoded protein to aromatic chemicals is indistinguishable from that produced from the wild type *dmpR* gene (Shingler et al, *supra*).

pRS551 (Simons, R. W., F. Houman, and N. Kleckner. 1987. "Improved single and multicopy *lac*-based cloning vectors for protein and operon fusions," Gene **53**:85-96.), a promoter assay vector, contains homology to the engineered *trp* operon of strain TE2680 and thus, allows integration of promoter-*lacZ* fusions into the *E. coli*

chromosome. pAW51 is a derivative of pRS551 that carries the *dmp* operon promoter *Pdmp* on a 0.6 kb DNA fragment fused to the pRS551 *lacZ* reporter gene.

pAW50 was derived from pBR322 (New England Biolabs, Beverly, MA). Following removal of the pBR322 *Nde*I site, a 2.4 kb *Not*I fragment containing *dmpR wt-N* was cloned into a *Not*I linker which replaced the *Sca*I site normally located in the ampicillin resistance gene of pBR322. An *Eco*RI restriction digest followed by ligation removed the promoter of the ampicillin resistance gene, as well as the 5' *Not*I site. pAW50 contains *dmpR* sequences extending approximately 650 base pairs upstream from the *dmpR* translation initiation site.

B. Genetic techniques. Plasmid DNA was isolated using a Qiagen Plasmid Kit (Qiagen, Inc., Chatsworth, CA) or by a mini-prep alkaline lysis method (Lee, S-y, and S. Rasheed. 1990. "A simple procedure for maximum yield of high-quality plasmid DNA," *Biotechniques* **9**:676-679.). Standard methods were used for restriction digests, gel electrophoresis and ligations. Transformation of *E. coli* was done by the electroporation method (Dower, W. J., J. F. Miller, and C. W. Ragsdale. 1988. "High efficiency transformation of *E. coli* by high voltage electroporation," *Nucleic Acids Res.* **16**:6127-6145.) using a Bio-Rad Gene Pulser II unit (Bio-Rad, Hercules, CA). Standard PCR to amplify the Po fragment was done as described by Innes et al. (Innes, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White. 1990. PCR Protocols: a Guide to Methods and Applications, Academic Press, New York.).

pVI401 (See, e.g., Shingler and Moore, *supra*) served as the template for amplifying Po in a reaction that included primers *Pdmp*5'-*Eco*RI (5'-CCATCGCTGAATTCTGCAGCAACAG-3'), SEQ ID No. 14 hereof, and *Pdmp*3'-*Bam*HI (5'-CGCACACGGATCCAACGAGTGAG-3'), SEQ ID No. 15 hereof. Primers were synthesized on an Applied Biosystems DNA/RNA Synthesizer 394 (Applied Biosystems, Inc. Foster City, CA) in the DNA synthesis laboratory of the Life Sciences Division at LANL. PCR was carried out on a Perkin-Elmer 9600 thermal cycler with a 2 minute denaturation step at 92° C followed by 25 cycles of one minute each at the following temperatures: 92° C, 52° C, and 72° C. The Po PCR product was digested with *Bam*HI and *Eco*RI to allow directed cloning in front of the promoterless *lacZ* gene of pRS551 for creation of the *Pdmp-lacZ* fusion of pAW51.

Mutagenic PCR to change the DmpR sensor domain was done by a modification of Cadwell and Joyce's method (Cadwell, R. C., and G. F. Joyce. 1995. Mutagenic PCR, p. 583-589. In C. W. Dieffenbach and G. S. Dveksler (ed.), PCR Primer, A Laboratory Manual. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, N.Y.).

5 pAW50 served as template in the mutagenic PCR reaction with 25 pmoles each of the following primers: dmpR5'-75 (5'-GCCGTCGATTGATCATTTGG-3'), SEQ ID No. 16 hereof, and dmpR3'-976, (5'-TGTCCATCATATTGCGCACG-3'), SEQ ID No. 17 hereof. In addition, the reaction contained 5 mM MgCl₂, 0.5 mM MnCl₂, 0.2 mM dATP and dGTP, 0.8 mM dCTP and dTTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.001% (wt/vol)
10 gelatin, and 5 units of AmpliTaq polymerase (Perkin Elmer, Foster City, CA). The mutagenic PCR amplification cycle followed a 2 min. denaturation at 92° C and consisted of 30 cycles of 94° C (10 s), 56° C (20 s) and 72° C (1 min.).

pAW50 and the mutagenized PCR products were each digested with *Nde*I and *Sac*II. The 525 base pair *Nde*I-*Sac*II PCR fragment contained most of the *dmpR* sensor domain. This fragment and pAW50, excluding the wild type sensor domain, were gel
15 purified from low melting point agarose using Elutip-D columns (Schleicher and Schell, Keene, NH). The purified DNA fragments served as components in a ligation reaction to reassemble pAW50 derivatives carrying *dmpR* with variously mutated sensor regions.

C. Test strain construction and screen for sensor domain mutations.

20 pAW51, carrying the *Pdmp-lacZ* fusion, was linearized through restriction with *Scal*, which cuts at a single site within the vector ampicillin resistance gene. The linearized plasmid was then used to transform TE2680 to kanamycin resistance. Transformants were screened for loss of ampicillin and chloramphenicol resistance, a condition indicating integration of the *Pdmp-lacZ* fusion into the TE2680 chromosome at the *trp*
25 operon. The general transducing phage P1*kc* (American Type Culture Collection, Rockville, CA) was used to transfer the fusion to the chromosome of MC4100 resulting in strain AW101.

AW101 was used as a test strain to identify and characterize changes in DmpR's sensing capacity subsequent to sensor domain mutagenesis. pAW50 derivatives were
30 electroporated into AW101 and transformants were selected on Luria-Bertani (Difco, Detroit, MI) plates containing 10.5 µg/ml tetracycline. Transformants were then replica-

plated onto M9 minimal medium (10) plates containing 0.2% glucose, 30 µg/ml tryptophan, 1 µg/ml thiamine, 10.5 µg/ml tetracycline, 0.25% 5-bromo-4-chloro-3-indoyl-β-D-galactoside (X-Gal), and either no inducer (NI) or 0.05 mM of a phenol derivative. Cells that formed blue colonies on plates containing a phenol derivative were subject to
 5 liquid β-galactosidase assays.

D. β-Galactosidase Assays. Overnight cultures of AW101 carrying pAW50 derivatives were diluted 1000-fold into Luria-Bertani broth containing 10.5 µg/ml tetracycline. When cells reached an OD₅₉₅ nm between 0.60 and 0.90 as measured on a Lambda Bio uv/vis spectrophotometer (Perkin Elmer Corp. Analytical Instruments,
 10 Norwalk, CT), 500 µL samples were pelleted and immediately resuspended in 500 µL spent Luria-Bertani broth containing the appropriate phenol compound. Cell incubation was then continued with shaking at 37° C for 2 h. Samples were pelleted and frozen at -70° C for assay the following day.

Liquid β-galactosidase assays were performed using a modification of Miller's
 15 assay (Miller, J. H., 1972. "Experiments in Molecular Genetics," Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.). Cell sample pellets were thawed and resuspended in Z buffer. The optical density at 595 nm of each cell suspension was read in a microtiter plate using an automated microplate reader (BIO-TEK Instruments, Inc., Winooski, VT). Following the addition of 15 µL 0.1% sodium dodecyl sulfate and
 20 25 µL HCCl₃, the remaining cell suspension was vortexed for 30 s to lyse cells. The reaction was begun with the addition of 50 µL o-nitrophenyl-β-D-galactopyranoside (2.5 mg/ml) to the lysed cells. Reactions were incubated at 26° C until stopped with the addition of 50 µL 1M Na₂CO₃. Color development of the reactions was read at OD₄₁₅ on the microplate reader. Arbitrary units for graphing purposes were calculated as
 25 (1000 X OD₄₁₅)/(time)(OD₅₉₅) where time is the reaction time in minutes. Equivalent cell volumes were read for both optical densities.

E. DNA Sequencing. Mutations in the *dmpR* sensor domains carried by pAW50 derivatives were identified using an ABI PRISM Dye Terminator Cycle Sequencing kit and following the manufacturer's (Perkin-Elmer) protocol.
 30 Electrophoresis of sequencing reactions was carried out on 4% polyacrylamide gels

using an ABI 373A Stretch DNA Sequencer (Applied Biosystems, Inc., Foster City, CA). Analysis of mutant sensor domain DNA and amino acid sequences was done using DNASTAR LASERGENE software (DNASTAR Inc., Madison, WI).

Figure 2a shows the results of a β -galactosidase assay for the detection of 2-chlorophenol, while FIG. 2b shows the assay for 2,4-dichlorophenol using the bacterial test strain containing either wild type DmpR or the derivative DmpR-B21 (SEQ ID No. 3 and No. 9 hereof), where NI indicates a negative control containing no inducer (no phenol). For all included graphs, units are β -galactosidase activity normalized for time length of assay and number of cells in assay. NI indicates a negative control containing no inducer (no phenol). Wild type DmpR has no apparent response to a 0.0025 mM solution of 2-chlorophenol (0.3 parts per million), whereas DmpR-B21 responds well with a 60-fold increase in β -galactosidase activity. 2-chlorophenol is a natural inducer of the wild type DmpR protein, as shown by its signal production (97 units) when exposed to a 0.025 mM solution of 2-chlorophenol. A more complex phenol, 2,4-dichlorophenol, elicits a response from DmpR-B21 at 0.025 mM (4 parts per million), but not from the natural DmpR protein. Note changes in axis between graphs.

Figure 3a shows assays comparing wild type DmpR and DmpR-B23 (SEQ ID No. 4 hereof) as detectors for six concentrations of 2-chlorophenol after a 60 minute exposure, while Fig. 3b shows assays comparing the response of wild type DmpR and DmpR-B17#2 (SEQ ID No. 2 hereof) to six concentrations of 4-chloro-3-methylphenol. Both DmpR-B23 and DmpR-B17#2 are significantly better detectors of substituted phenols than the wild type protein at all concentrations assayed.

Figure 4a shows that a four-hour exposure to phenols increases the detection signal (in β -galactosidase activity units) for low concentrations of 2-chlorophenol for DmpR-B23, whereas Fig. 4b shows the response of DmpR-B17#2 (SEQ ID No. 2 hereof) to low concentrations of 2,4-dichlorophenol, 2-nitrophenol, and 4-nitrophenol. DmpR-B23's response to 0.5 ppm 2-chlorophenol was more than 20-fold higher than that of the wild type DmpR protein. DmpR-B17#2 responds to low concentrations of phenols (2,4-dichlorophenol, 2-nitrophenol, and 4-nitrophenol) that are poor effectors of the wild type DmpR protein.

Figure 5a compares the detection of 2,4-dichlorophenol by DmpR-B9 (SEQ ID No. 1 hereof) with that for unsubstituted phenol shown in Fig. 5b in contaminated soil, unsubstituted phenol being a natural effector of wild type DmpR. Detection of chemicals in contaminated soil is often difficult because soil may bind the phenols, making them less available to the test bacteria. However, DmpR-B9's capacity to detect phenols in soil remains significantly better than that of the wild type protein. The response of DmpR-B9 to 4 parts per million (ppm) 2,4-dichlorophenol in soil is six-fold higher than that of wild type DmpR. DmpR-B9's response to 2.5 ppm phenol is four-fold better than that of wild type.

Figure 6a compares the detection of 2,4-dichlorophenol, 2,4-dimethylphenol, and 4-nitrophenol by wild type DmpR with that for the engineered protein DmpR-B31 (SEQ ID No. 5 hereof), while Fig. 6b compares the detection of 2,4-dichlorophenol and 4-chloro-3-methylphenol by the wild type DmpR and DmpR-D12 (SEQ ID No. 7 hereof). DmpR-B31 shows a strong response to most substituted phenols, but also has a relatively high uninduced (NI) value. DmpR-D12's detection of 10 ppm 4-chloro-3-methylphenol and 3 ppm 2,4-dichlorophenol are eight and 10-fold higher than that of wild type DmpR.

Figure 7a compares the detection of 2-chlorophenol by DmpR-D9 (SEQ ID No. 6) with its detection by wild type DmpR, while Fig. 7b compares the detection of 2-nitrophenol by DmpR-D9 with that for wild type DmpR. DmpR-D9's response to 2-chlorophenol is nineteen-fold higher than that of the wild type protein, while its response to 2-nitrophenol is five times higher than that of wild type DmpR.

The foregoing description of the invention has been presented for purposes of illustration and description and is not intended to be exhaustive or to limit the invention to the precise form disclosed, and obviously many modifications and variations are possible in light of the above teaching. For example, it would be apparent to one having ordinary skill in the art after reading the present disclosure that mutating the sensor domain to enhance the response of bacteria to selected organic molecules could be achieved by removing the sensor domain from the bacterial DNA encoding the regulatory protein, subjecting the removed sensor domain to gene reshuffling, ligating the mutated sensor domain into the DNA encoding the regulatory protein, and testing

5

(1) GENERAL INFORMATION:

NUMBER OF SEQUENCES: 17

(2) INFORMATION FOR SEQ ID NO. 1: (DMPR-B9)

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 540 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA fragment

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Pseudomonas CF600*

(xi) SEQUENCE DESCRIPTION: SEQ. ID. NO. 1 (B9):

ATGCCGATCG	AGTACAAGCC	TGAAATCCAG	CACTCCGATT	TCAAGGACCT	GACCAACCTG	60
ATCCACTTCC	AGAGCATGGA	AGGCAAGATC	TGGCTTGCG	AACAGCGCAT	GCTGTTGCTG	120
CAGTCTTCAG	CGATGGCCAG	CTTTCGCCGG	GAAATGGTCA	ATACCCTGGG	CATCGAACGC	180
GCCAAGGGCT	TGTTCTGCG	CCATGGTTAC	CAGTCCGGCC	TGAAGGATGC	CGAACTGGCC	240
AGGAAGCTGA	GACCGAATGC	CAGCGAAGTC	GGCATGTTCC	TCGCTGGGCC	GCAGATGCAT	300
TCACTCAAGG	GTCTGGTCAA	GGTCCGCCCC	ACCGAGCTCG	ATATCGACAA	GGAATACGGG	360
CGCTTCTATG	CCGAGATGGA	GTGGATCGAC	TGGTTCGAGG	TGGAAATCTG	CCAGACCGAC	420
CTGGGGCAGA	TGCAAGACCC	GGTGTGCTGG	ACTGTGCTCG	GCTACGCCTG	CGCCTATTCC	480
TCGGCGTTCA	TGGGCCGGGA	AATCATCTTC	AAGGAAGTCA	GCTGCCGCGG	CTGCGGCGGC	540

(2) INFORMATION FOR SEQ ID NO. 2: (DMPR-B17#2)

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 540 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA fragment

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Pseudomonas CF600*

(xi) SEQUENCE DESCRIPTION: SEQ. ID. NO. 2 (B17#2):

ATGCCGATCA AGTACAAGCC TGAAATCCAG CACTCCGATT TCAAGGACCT GACCAACCTG	60
ATCCACTTCC AGAGCATGGA AGGCAAGATC TGGCTTGGCG AACAGCGCAT GCTGTTGCTG	120
CAGTTTTTCGG CGATGGCCAG CTTTCGCCGG GAAATGGTCA ATACCCTGGG CATCGAACGC	180
GCCAAGGGCT TGTTCTGCG CCATGGTTAC CAGTCCGGCC TGAAGGATGC CGAACTGGCC	240
AGGAAGCTGA GACCGAATGC CAGCGAAGTC GGCATGTTCC TCGCTGGGCC GCAGATGCAT	300
TCACTCAAGG GTCTGGTCAA GGTCCGCCCC ACCGAGCTCG ATATCGACAA GGAATACGGG	360
CGCTTCTATG CCGAGATGGA GTGGATCGAC TGGTTCGAGG TGGAAATCTG CCAGACCGAC	420
CTGGGGCAGA TGCAAGACCC GGTGTGCTGG ACTGTGCTCG GCTACGCCTG CGCCTATTCC	480
TCGGCGTTCA TGGGCCGGGA AATCATCTTC AAGGAAGTCA GCTGCCGCGG CTGCGGCGGC	540

(2) INFORMATION FOR SEQ ID NO. 3: (DMPR-B21)

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 540 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA fragment

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Pseudomonas CF600*

(xi) SEQUENCE DESCRIPTION: SEQ. ID. NO. 3 (B21):

```

ATGCCGATCA AGTACAAGCC TGAAATCCAG CACTCCGATT TCAAGGACCT GACCAACCTG    60
ATCCACTTCC AGAGCATGGA AGGCAAGATC TGGCTTGGCG AACACGCAT GCTGTTGCTG    120
CAGTTTTTCAG CGATGGCCAG CTTTCGCCGG GAAATGGTCA ATACCCTGGG CATCGAACGC    180
GCCAAGGGCT TGTTCTGCG CCATGGTTAC CAGTCCGGCC TGAAGGATGC CGAACTGGCC    240
AGGAAGCTGA GACCGAATGC CAGCGAAGTC GGCATGTTCC TCGCTGGGCC GCAGATGCAT    300
TCACTCAAGG GTCTGGTCAA GGTCCGCCCC ACCGGGCTCG ATATCGACAA GGAATACGGG    360
CGCTTCTATG CCGAGATGGA GTGGATCGAC TGGTTCGAGG TGGAAATCTG CCAGACCGAC    420
CTGGGGCAGA TGCAAGACCC GGTGTGCTGG ACTGTGCTCG GCTACGCCTG CGCCTATTCC    480
TCGGCGTTCA TGGGCCGGGA AATCATCTTC AAGGAAGTCA GCTGCCGCGG CTGCGGCGGC    540

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(2) INFORMATION FOR SEQ ID NO. 4: (DMPR-B23)

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 540 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA fragment

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Pseudomonas CF600*

(xi) SEQUENCE DESCRIPTION: SEQ. ID. NO. 4 (B23):

```

ATGCCGATCA AGTACAAGCC TGAAATCCGG CACTCCGATT TCAAGGACCT GACCAACCTG    60
ATCCACTTCC AGAGCATGGA AGGCAAGATC TGGCTTGGCG AACAGCGCAT GCTGTTGCTG    120
CAGTTTTTCAG CGATGGCCAG CTTTCGCCGG GAAATGGTCA ATACCCTGGG CATCGAACGC    180
GCCAAGGGCT TGTTCTGCG CCATGGTTAC CAGTCCGGCC TGAAGGATGC CGAACTGGCC    240
AGGAAGCTGA GACCGAATGC CAGCGAAGTC GGCATGTTCC TCGCTGGGCC GCAGATGCAT    300
TCACTCAAGG GTCTGGTCAA GGTCCGCCCC ACCGAGCTCG ATATCGACAT GGAATACGGG    360
CGCTTCTATG CCGAGATGGA GTGGATCGAC TGGTTCGAGG TGGAAATCTG CCAGACCGAC    420

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CTGGGGCAGA TGCAAGACCC GGTGTGCTGG ACTGTGCTCG GCTACGCCTG CGCCTATTCC 480
 TCGGCGTTCA TGGGCCGGGA AATCATCTTC AAGGAAGTCA GCTGCCGCGG CTGCGGCGGC 540

(2) INFORMATION FOR SEQ ID NO. 5: (DMPR-B31)

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 540 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA fragment

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Pseudomonas CF600*

(xi) SEQUENCE DESCRIPTION: SEQ. ID. NO. 5 (B31):

ATGCCGATCA AGTACAAGCC TGAAATCCAG CACTCCGATT TCAAGGACCT GACCAACCTG 60
 ATCCACTTCC AGAGCATGGA AGGCAAGATC TGGCTTGGCG AACAGCGCAT GCTGTTGCTG 120
 CAGTTTTCAG CGATGGCCAG CTTTCGCCGG GAAATGGTCA ATACCCTGGG CGTCGAACGC 180
 ACCAAGGGCT TGTTCTTGCG CCATGGTTAC CAGTCCGGCC TGAAGGATGC CGAACTGGCC 240
 AGGAAGCTGA GACCGAATGC CAGCGAAGTC GGCATGTTCC TTGCTGGGCC GCAGATGCAT 300
 TCACTCAAGG GTCTGGTCAA GGTCCGCCCC ACCGAGCTCG ATATCGACAA GGAATACGGG 360
 CGCTTCTATG CCGAGATGGA GTGGATCGAC TGGTTCGAGG TGGAAATCTG CCAGACCGAC 420
 CTGGGGCAGA TGCAAGGCCG GGTGTGCTGG ACTGTGCTCG GCTACGCCTG CGCCTATTCC 480
 TCGGCGTTCA TGGGCCGGGA AATCATCTTC AAGGAAGTCA GCTGCCGCGG CTGCGGCGGC 540

(2) INFORMATION FOR SEQ ID NO. 6: (DMPR-D9)

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 540 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA fragment

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Pseudomonas CF600*

(xi) SEQUENCE DESCRIPTION: SEQ. ID. NO. 6 (D9):

ATGCCGATCA	AGTACAAGCC	TGAAATCCAG	CACTCCGATT	TCAAGGACCT	GACCAACCTG	60
ATCCACTTCC	AGAGCATGGA	AGGCAAGATC	TGGCTTGGCG	AACAGCGCAT	GCTGTTGCTG	120
CAGTTTTTCAG	CGATGGCCAG	CTTCCGCCGG	GAAATGGTCA	ATACCCTGGG	CATCGAACGC	180
GCCAAGGGCT	TGTTCTGCG	CCATGGTTAC	CAGTCCGGCC	TGAAGGATGC	CGAACTGGCC	240
AGGAAGCTGA	GACCGAATGC	CAGCGAAGTC	GGCATGTTCC	TCGCTGGGCC	GCAGATGCAT	300
TCACTCAAGG	GTCTGGTCAA	GGTCCGCCCC	ACCGAGCTCG	ATATCGGCAG	GGAATACGGG	360
CGCTTCTATG	CCGAGATGGA	GTGGATCGAC	TGGTTCGAGG	TGGAAATCTG	CCAGACCGAC	420
CTGGGGCAGA	TGCAAGACCC	GGTGTGCTGG	ACTGTGCTCG	GCTACGCCTG	CGCCTATTCC	480
TCGGCGCTCA	TGGGCCGGGA	AATCATCTTC	AAGGAAGTCA	GCTGCCGCGG	CTGCGGCGGC	540

(2) INFORMATION FOR SEQ ID NO. 7: (DMPR-D12)

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 540 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA fragment

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Pseudomonas CF600*

(xi) SEQUENCE DESCRIPTION: SEQ. ID. NO. 7 (D12):

ATGCCGATCA	AGTACAAGCC	TGAAATCCAG	CACTCCGATT	TCAAGGACCT	GACCAACCTG	60
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ATCCACTTCC AGAGCATGGA AGGCAAGATC TGGCTTGGCG AACAGCGCAT GCTGTTGCTG 120
 CAGTTTTTCAG CGATGGCCAG CTTCCGCCGG GAAATGGTCA ATACCCTGGG CATCGAACGC 180
 GCCAAGGGCT TGTTCCTGCG CCATGGTTAC CAGTCCGGCC TGAAGGATGC CGAACTGGCC 240
 AGGAAGCTGA GACCGAATGC CAGCGAAGTC GGCATGTTCC TCGCTGGGCC GCAGATGCAT 300
 TCACTCAAGG GTCTGGTCAA GGTCCGCCCC ACCGAGCTCG ATATCGACAA GGAATACGGG 360
 CGCTTCTATG CCGAGATGGA GTGGATCGAC TGGTTCGAGG TGGAAATCTG CCAGACCGAC 420
 CCGGGGCAGA TGCAAGACCC GGTGTGCTGG ACTGTGCTCG GCTACGCCTG CGCCTATTCC 480
 TCGGCGTTCA TGGGCCGGGA AATCATCTTC AAGGAAGTCA GCTGCCGCGG CTGCGGCGGC 540

(2) INFORMATION FOR SEQ ID NO. 8 (B9):

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 180 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein fragment

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Pseudomonas CF600*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 8 (B9):

Met	Pro	Ile	Lys	Tyr	Glu	Pro	Glu	Ile	Gln	His	Ser	Asp	Phe	Lys	Asp	1	5	10	15
Leu	Thr	Asn	Leu	Ile	His	Phe	Gln	Ser	Met	Glu	Gly	Lys	Ile	Trp	Leu	20	25	30	
Gly	Glu	Gln	Arg	Met	Leu	Leu	Leu	Gln	Ser	Ser	Ala	Met	Ala	Ser	Phe	35	40	45	
Arg	Arg	Glu	Met	Val	Asn	Thr	Leu	Gly	Ile	Glu	Arg	Ala	Lys	Gly	Leu	50	55	60	
Phe	Leu	Arg	His	Gly	Tyr	Gln	Ser	Gly	Leu	Lys	Asp	Ala	Glu	Leu	Ala	65	70	75	80
Arg	Lys	Leu	Arg	Pro	Asn	Ala	Ser	Glu	Val	Gly	Met	Phe	Leu	Ala	Gly	85	90	95	

[illegible]

(2) INFORMATION FOR SEQ ID NO. 9 (B21):

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 180 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein fragment

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Pseudomonas* CF600

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 9 (B21):

Met	Pro	Ile	Lys	Tyr	Lys	Pro	Glu	Ile	Gln	His	Ser	Asp	Phe	Lys	Asp
1				5					10					15	
Leu	Thr	Asn	Leu	Ile	His	Phe	Gln	Ser	Met	Glu	Gly	Lys	Ile	Trp	Leu
			20					25					30		
Gly	Glu	Gln	Arg	Met	Leu	Leu	Leu	Gln	Phe	Ser	Ala	Met	Ala	Ser	Phe
		35					40					45			
Arg	Arg	Glu	Met	Val	Asn	Thr	Leu	Gly	Ile	Glu	Arg	Ala	Lys	Gly	Leu
	50					55					60				
Phe	Leu	Arg	His	Gly	Tyr	Gln	Ser	Gly	Leu	Lys	Asp	Ala	Glu	Leu	Ala
65					70					75					80
Arg	Lys	Leu	Arg	Pro	Asn	Ala	Ser	Glu	Val	Gly	Met	Phe	Leu	Ala	Gly
				85					90					95	

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Pro Gln Met His Ser Leu Lys Gly Leu Val Lys Val Arg Pro Thr Gly
      100                      105                      110

Leu Asp Ile Asp Lys Glu Tyr Gly Arg Phe Tyr Ala Glu Met Glu Trp
      115                      120                      125

Ile Asp Ser Phe Glu Val Glu Ile Cys Gln Thr Asp Leu Gly Gln Met
      130                      135                      140

Gln Asp Pro Val Cys Trp Thr Leu Leu Gly Tyr Ala Cys Ala Tyr Ser
      145                      150                      155                      160

Ser Ala Phe Met Gly Arg Glu Ile Ile Phe Lys Glu Val Ser Cys Arg
      165                      170                      175

Gly Cys Gly Gly
      180

```

(2) INFORMATION FOR SEQ ID NO. 10 (B23):

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 180 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein fragment

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Pseudomonas CF600*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 10 (B23):

```

Met Pro Ile Lys Tyr Lys Pro Glu Ile Arg His Ser Asp Phe Lys Asp
 1                      5                      10                      15

Leu Thr Asn Leu Ile His Phe Gln Ser Met Glu Gly Lys Ile Trp Leu
      20                      25                      30

Gly Glu Gln Arg Met Leu Leu Leu Gln Phe Ser Ala Met Ala Ser Phe
      35                      40                      45

Arg Arg Glu Met Val Asn Thr Leu Gly Ile Glu Arg Ala Lys Gly Leu
      50                      55                      60

Phe Leu Arg His Gly Tyr Gln Ser Gly Leu Lys Asp Ala Glu Leu Ala
      65                      70                      75                      80

Arg Lys Leu Arg Pro Asn Ala Ser Glu Val Gly Met Phe Leu Ala Gly
      85                      90                      95

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Pro	Gln	Met	His	Ser	Leu	Lys	Gly	Leu	Val	Lys	Val	Arg	Pro	Thr	Glu
100				105				110							
Leu	Asp	Ile	Asp	Met	Glu	Tyr	Gly	Arg	Phe	Tyr	Ala	Glu	Met	Glu	Trp
115				120				125							
Ile	Asp	Ser	Phe	Glu	Val	Glu	Ile	Cys	Gln	Thr	Asp	Leu	Gly	Gln	Met
130				135				140							
Gln	Asp	Pro	Val	Cys	Trp	Thr	Leu	Leu	Gly	Tyr	Ala	Cys	Ala	Tyr	Ser
145				150				155				160			
Ser	Ala	Phe	Met	Gly	Arg	Glu	Ile	Ile	Phe	Lys	Glu	Val	Ser	Cys	Arg
165				170				175							
Gly	Cys	Gly	Gly												
180															

(2) INFORMATION FOR SEQ ID NO. 11 (B31):

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 180 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein fragment

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Pseudomonas* CF600

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 11(B31):

Met	Pro	Ile	Lys	Tyr	Lys	Pro	Glu	Ile	Gln	His	Ser	Asp	Phe	Lys	Asp
1				5					10					15	
Leu	Thr	Asn	Leu	Ile	His	Phe	Gln	Ser	Met	Glu	Gly	Lys	Ile	Trp	Leu
			20					25					30		
Gly	Glu	Gln	Arg	Met	Leu	Leu	Leu	Gln	Phe	Ser	Ala	Met	Ala	Ser	Phe
		35					40					45			
Arg	Arg	Glu	Met	Val	Asn	Thr	Leu	Gly	Val	Glu	Arg	Thr	Lys	Gly	Leu
	50					55					60				
Phe	Leu	Arg	His	Gly	Tyr	Gln	Ser	Gly	Leu	Lys	Asp	Ala	Glu	Leu	Ala
65					70					75					80
Arg	Lys	Leu	Arg	Pro	Asn	Ala	Ser	Glu	Val	Gly	Met	Phe	Leu	Ala	Gly
				85					90					95	
Pro	Gln	Met	His	Ser	Leu	Lys	Gly	Leu	Val	Lys	Val	Arg	Pro	Thr	Glu

[illegible]

(2) INFORMATION FOR SEQ ID NO. 12 (D9):

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 180 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein fragment

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Pseudomonas* CF600

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 12 (D9):

Met	Pro	Ile	Lys	Tyr	Lys	Pro	Glu	Ile	Gln	His	Ser	Asp	Phe	Lys	Asp
1				5					10					15	
Leu	Thr	Asn	Leu	Ile	His	Phe	Gln	Ser	Met	Glu	Gly	Lys	Ile	Trp	Leu
			20					25					30		
Gly	Glu	Gln	Arg	Met	Leu	Leu	Leu	Gln	Phe	Ser	Ala	Met	Ala	Ser	Phe
		35					40					45			
Arg	Arg	Glu	Met	Val	Asn	Thr	Leu	Gly	Ile	Glu	Arg	Ala	Lys	Gly	Leu
	50					55					60				
Phe	Leu	Arg	His	Gly	Tyr	Gln	Ser	Gly	Leu	Lys	Asp	Ala	Glu	Leu	Ala
65					70					75					80
Arg	Lys	Leu	Arg	Pro	Asn	Ala	Ser	Glu	Val	Gly	Met	Phe	Leu	Ala	Gly
				85					90					95	
Pro	Gln	Met	His	Ser	Leu	Lys	Gly	Leu	Val	Lys	Val	Arg	Pro	Thr	Glu
			100					105					110		

Leu Asp Ile Gly Arg Glu Tyr Gly Arg Phe Tyr Ala Glu Met Glu Trp
 115 120 125
 Ile Asp Ser Phe Glu Val Glu Ile Cys Gln Thr Asp Leu Gly Gln Met
 130 135 140
 Gln Asp Pro Val Cys Trp Thr Leu Leu Gly Tyr Ala Cys Ala Tyr Ser
 145 150 155 160
 Ser Ala Leu Met Gly Arg Glu Ile Ile Phe Lys Glu Val Ser Cys Arg
 165 170 175
 Gly Cys Gly Gly
 180

(2) INFORMATION FOR SEQ ID NO. 13 (D12):

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 180 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein fragment

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Pseudomonas CF600*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 13 (D12):

Met Pro Ile Lys Tyr Lys Pro Glu Ile Gln His Ser Asp Phe Lys Asp
 1 5 10 15
 Leu Thr Asn Leu Ile His Phe Gln Ser Met Glu Gly Lys Ile Trp Leu
 20 25 30
 Gly Glu Gln Arg Met Leu Leu Leu Gln Phe Ser Ala Met Ala Ser Phe
 35 40 45
 Arg Arg Glu Met Val Asn Thr Leu Gly Ile Glu Arg Ala Lys Gly Leu
 50 55 60
 Phe Leu Arg His Gly Tyr Gln Ser Gly Leu Lys Asp Ala Glu Leu Ala
 65 70 75 80
 Arg Lys Leu Arg Pro Asn Ala Ser Glu Val Gly Met Phe Leu Ala Gly
 85 90 95
 Pro Gln Met His Ser Leu Lys Gly Leu Val Lys Val Arg Pro Thr Glu
 100 105 110

[illegible]

(2) INFORMATION FOR SEQ ID NO. 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA fragment

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Pseudomonas* CF600

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 14:

CCATCGCTGA ATTCTGCAGC AACAG

(2) INFORMATION FOR SEQ ID NO. 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA fragment

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Pseudomonas CF600*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 15:

CGCACACGGA TCCAACGAGT GAG

(2) INFORMATION FOR SEQ ID NO. 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA fragment

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Pseudomonas CF600*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 16:

CCGTCGATTG ATCATTGG

(2) INFORMATION FOR SEQ ID NO. 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA fragment

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Pseudomonas CF600*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 17:

TGTCCATCAT ATTGCGCACG

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WHAT IS CLAIMED IS:

1. A method for enhancing the response of bacteria to organic molecules, said bacteria having a regulatory protein with discrete functional domains for independent activities, one such domain being a sensor domain that detects said organic molecules through a direct physical interaction forming a protein-molecule
- 5 complex which binds to a cognate promoter sequence and activates expression of genes encoding metabolic enzymes, said method comprising modifying the sensor domain of the regulatory protein such that the response to the organic molecule is enhanced without altering the other domains.
2. The method as described in claim 1, wherein the bacteria are selected from the group consisting of *Pseudomonas* and *Acinetobacter* bacteria.
3. The method as described in claim 1, wherein the regulatory protein is selected from the group consisting of DmpR, MopR, PhhR, PhlR, XylR, and TbuT.
4. The method as described in claim 1, wherein the step of modifying the sensor domain of the regulatory protein is achieved by mutating the sensor domain thereof.
5. The method as described in claim 4, wherein said step of mutating the sensor domain comprises the steps of removing the sensor domain from the bacterial DNA encoding the regulatory protein, subjecting the removed sensor domain to mutagenic polymerase chain reaction, ligating the mutated sensor domain into the DNA
- 5 encoding the regulatory protein, and testing the bacteria for enhanced response to said organic molecules.
6. The method as described in claim 4, wherein said step of mutating the sensor domain comprises the steps of removing the sensor domain from the bacterial DNA encoding the regulatory protein, subjecting the removed sensor domain to gene reshuffling, ligating the mutated sensor domain into the DNA encoding the regulatory
- 5 protein, and testing the bacteria for enhanced response to said organic molecules.
7. The method as described in claim 1, wherein said organic molecules are selected from the group consisting of phenols and substituted phenols.

ABSTRACT

Detection of phenols using engineered bacteria. A biosensor can be created by placing a reporter gene under control of an inducible promoter. The reporter gene produces a signal when a cognate transcriptional activator senses the inducing chemical. Creation of bacterial biosensors is currently restricted by limited knowledge of the genetic systems of bacteria that catabolize xenobiotics. By using mutagenic PCR to change the chemical specificity of the *Pseudomonas* species CF600 DmpR protein, the potential for engineering novel biosensors for detection of phenols has been demonstrated. DmpR, a well-characterized transcriptional activator of the *P. CF600*'s *dmp* operon mediates growth on simple phenols. Transcription from *Po*, the promoter heading the *dmp* operon, is activated when the sensor domain of DmpR interacts with phenol and mono-substituted phenols. By altering the sensor domain of the DmpR, a group of DmpR derivatives that activate transcription of a *Po-lacZ* fusion in response to eight of the EPA's eleven priority pollutant phenols has been created. The assays and the sensor domain mutations that alter the chemical specificity of DmpR is described.

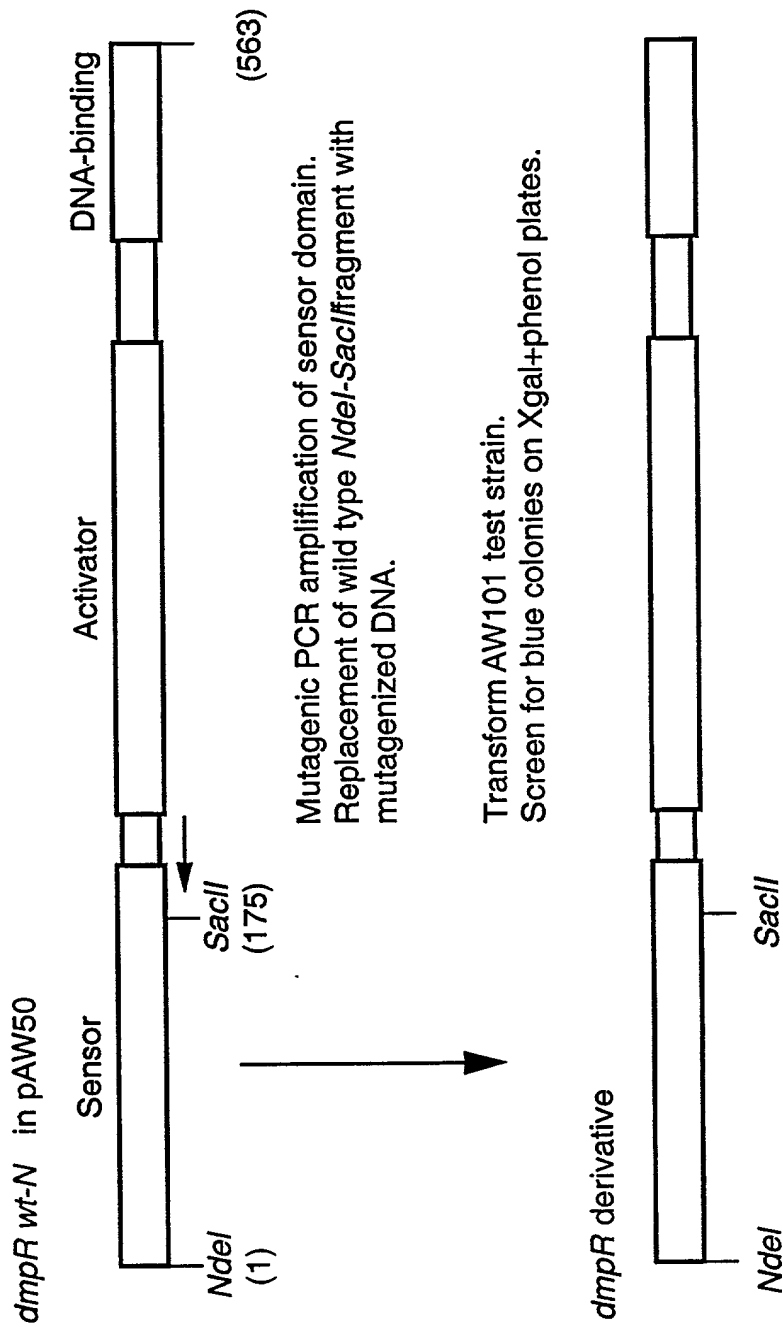


Fig. 1

FIG. 3a

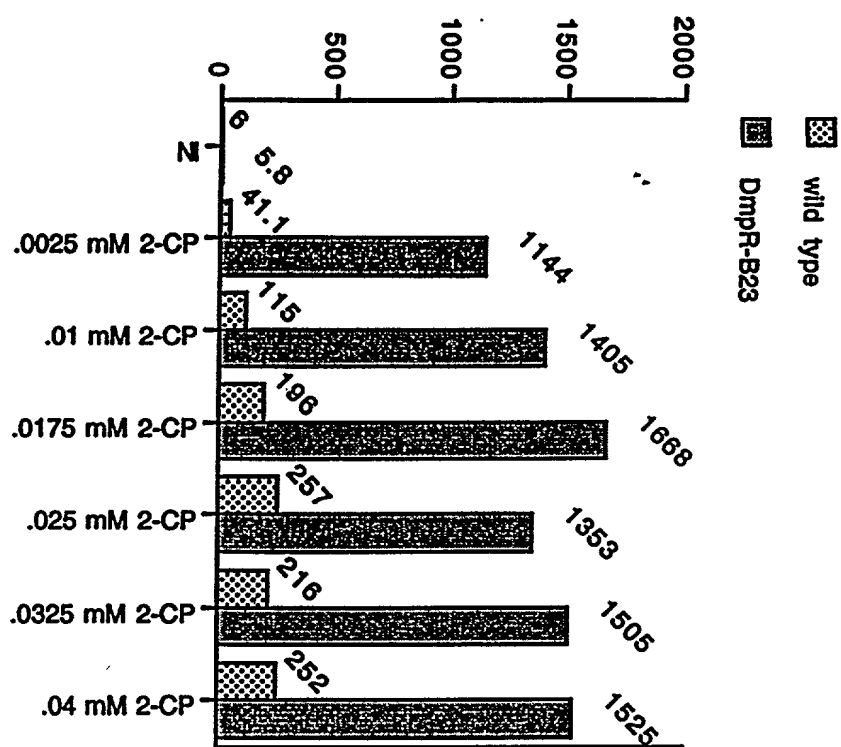
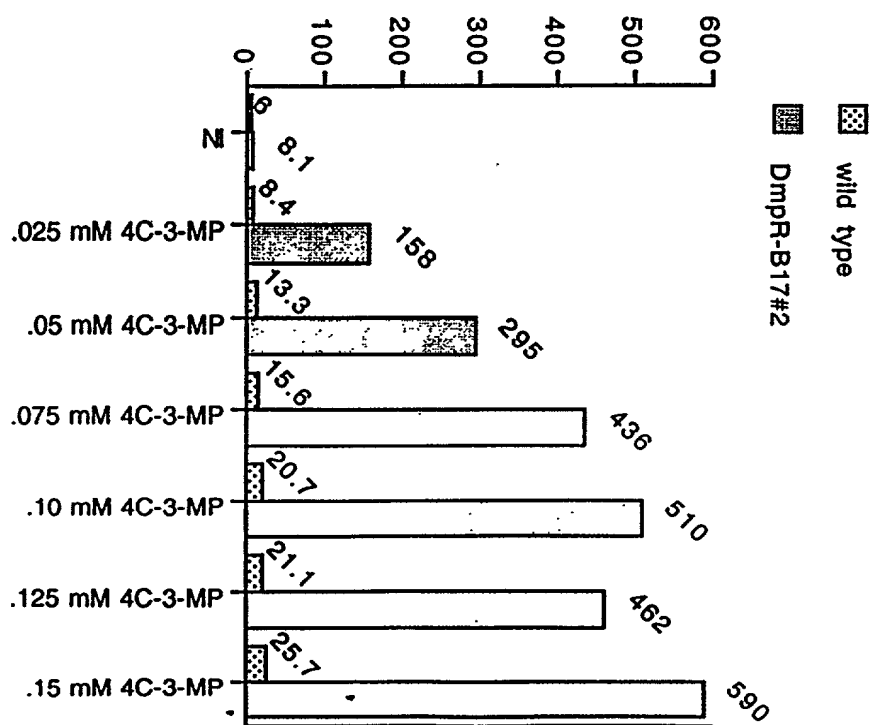


FIG. 3b



no phenol
2,4-dichlorophenol
parts per million

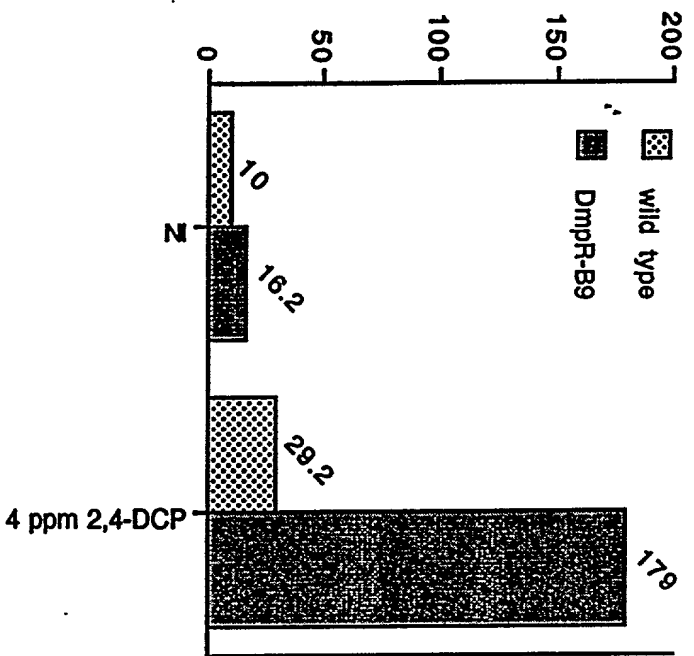


FIG. 5a

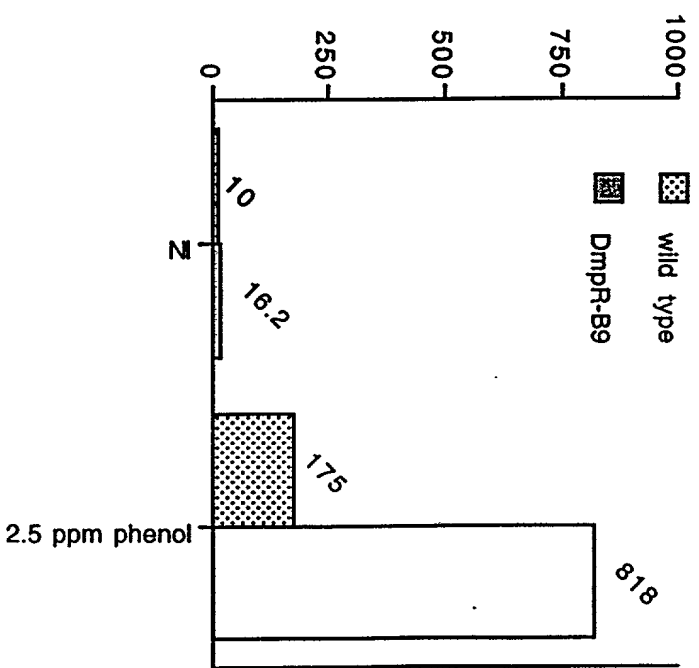


FIG. 5b

[illegible]

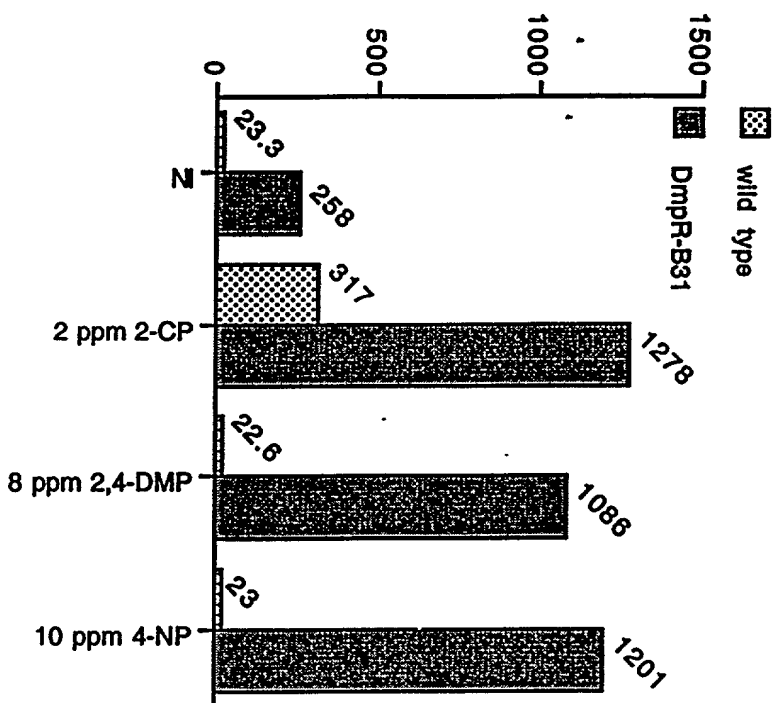


FIG. 6a

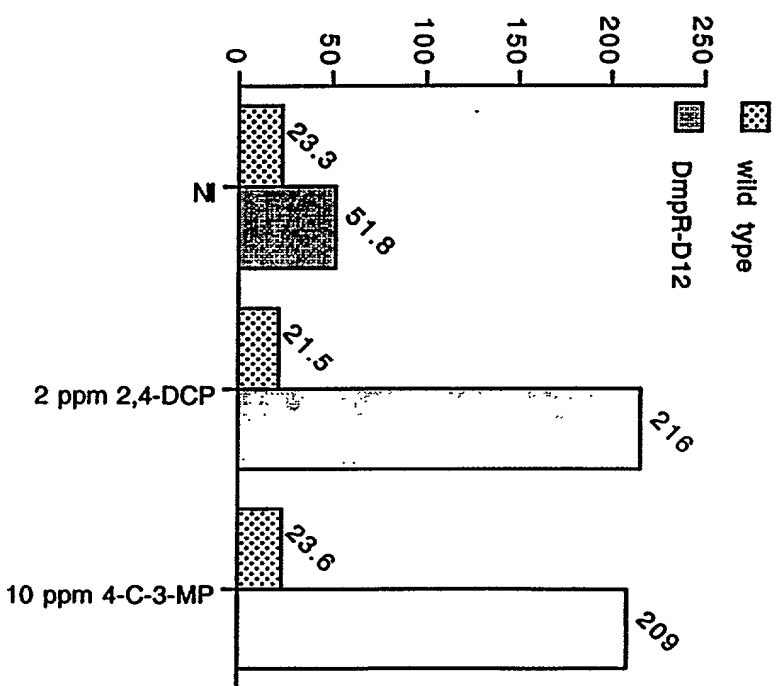


FIG. 6b

NI
2-CP
2,4-DCP
2,4-DMP
4-NP
4-C-3-MP

no phenol
2-chlorophenol
2,4-dichlorophenol
2,4-dimethylphenol
4-nitrophenol
4-chloro-3-methylphenol

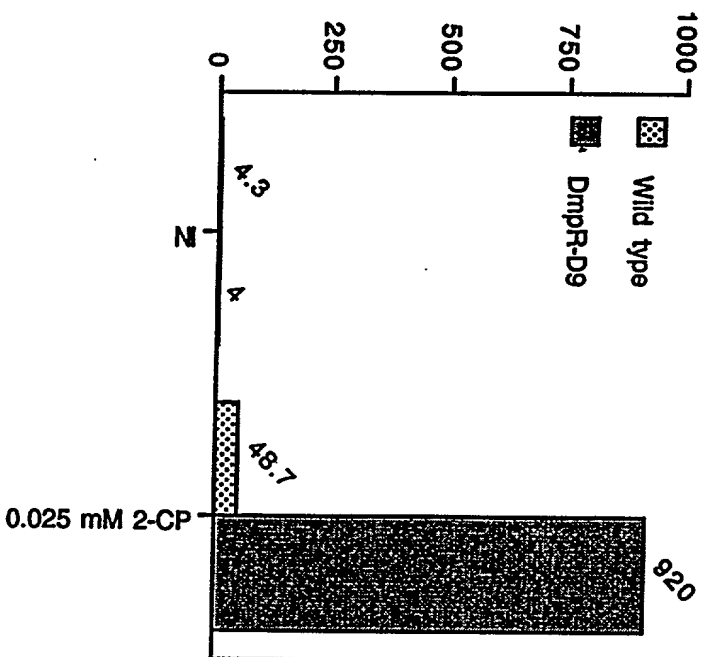


FIG. 7a

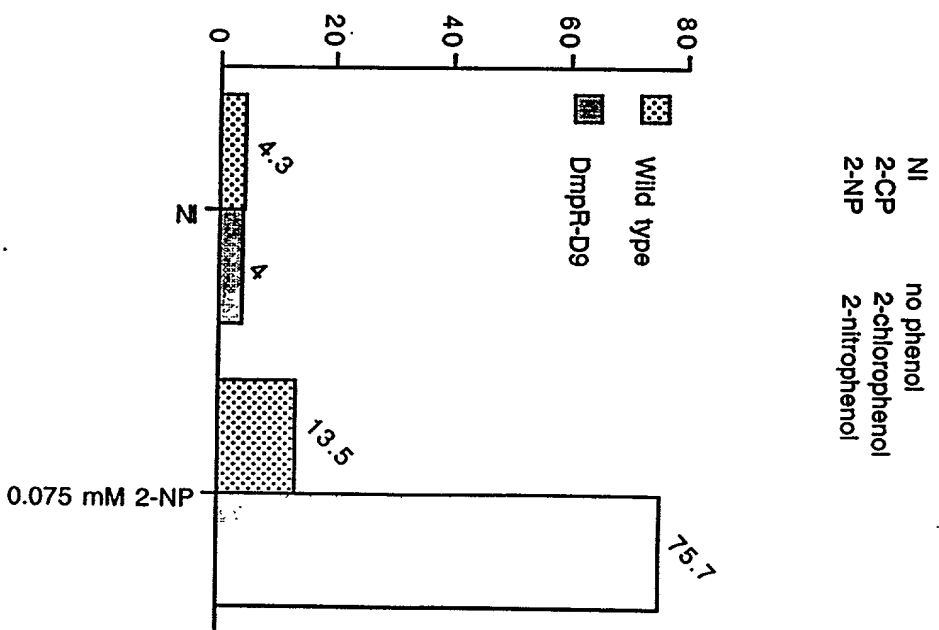


FIG. 7b